How I treat myelofibrosis

Ayalew Tefferi

How I treat myelofibrosis

Ayalew Tefferi[1]

[1]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN

It is currently assumed that myelofibrosis (MF) originates from acquired mutations that target the hematopoietic stem cell and induce dysregulation of kinase signaling, clonal myeloproliferation, and abnormal cytokine expression. These pathogenetic processes are interdependent and also individually contributory to disease phenotype–bone marrow stromal changes, extramedullary hematopoiesis, ineffective erythropoiesis, and constitutional symptoms. Molecular pathogenesis of MF is poorly understood despite a growing list of resident somatic mutations that are either functionally linked to Janus kinase (JAK)–signal transducer and activator of transcription hyperactivation (eg *JAK2*, *MPL*, and *LNK* mutations) or possibly involved in epigenetic dysregulation of transcription (*TET2*, *ASXL1*, or *EZH2* mutations). Current prognostication in primary MF is based on the Dynamic International Prognostic Scoring System-plus model, which uses 8 independent predictors of inferior survival to classify patients into low, intermediate 1, intermediate 2, and high-risk disease groups; corresponding median survivals are estimated at 15.4, 6.5, 2.9, and 1.3 years. Such information is used to plan a risk-adapted treatment strategy for the individual patient, which might include observation alone, conventional or investigational (eg, JAK inhibitors, pomalidomide) drug therapy, allogenic stem cell transplantation with reduced- or conventional-intensity conditioning, splenectomy, or radiotherapy. I discuss these treatment approaches in the context of who should get what and when. (*Blood*. 2011;117(13):3494-3504)

Terminology: What do I mean by myelofibrosis?

Bone marrow fibrosis, usually shown by silver (reticulin fibrosis) or trichrome (collagen fibrosis) stains, can accompany a number of hematologic and nonhematologic conditions, including myeloid or lymphoid neoplasms, metastatic cancer, autoimmune diseases, hyperparathyroidism, vitamin D deficiency, pulmonary hypertension, gray platelet syndrome, treatment with growth factors (eg, thrombopoietin agonists), hereditary thrombocytosis (eg, germline *MPL*S505N mutation), infections (eg, AIDS, leishmaniasis), and exposure to toxic substances (eg, thorium dioxide).[1] When describing such occurrences, I prefer to use the phrase "with bone marrow fibrosis" instead of "with myelofibrosis": for example, "myelodysplastic syndromes (MDSs) with bone marrow fibrosis" rather than "MDS with myelofibrosis." I use the term myelofibrosis (MF) exclusively in reference to the myeloproliferative neoplasm (MPN) that is classified by the World Health Organization (WHO) system as primary myelofibrosis (PMF),[2] or the phenotypically similar condition that develops in the setting of either polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF).[3] In addition, given our current understanding about the clonal nature of MF,[4] it is inaccurate to continue using alternative terms such as "*agnogenic* myeloid metaplasia" or "chronic *idiopathic* myelofibrosis."[5]

Classification: WHO all the way

The WHO classification system for hematopoietic tumors recognizes 5 categories of myeloid malignancies, including acute myeloid leukemia (AML), MDS, MPN, MDS/MPN overlap, and *PDGFR/FGFR1*-rearranged myeloid/lymphoid neoplasms with eosinophilia (Table 1).[2] The WHO MPN category includes 8 subcategories: PV, ET, PMF, chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, chronic eosinophilic leukemia not otherwise specified, mastocytosis, and MPN unclassifiable. The first 4 were originally described by William Dameshek as "myeloproliferative disorders" and are therefore currently referred to as "classic" MPN (Table 1).[6] "*BCR-ABL1*–negative MPN" is an operational term that is used in reference to PV, ET, and PMF.[7] Post-PV MF and post-ET MF are classified and diagnosed according to consensus criteria established by the International Working Group for MPN Research and Treatment (IWG-MRT).[3] The IWG-MRT also recommends the use of the term "blast phase PMF" when describing leukemic transformation.[3,5]

Pathogenesis: What do abnormal clones, mutations, and cytokines mean?

MPN are stem cell–derived monoclonal (or possibly oligoclonal) hemopathies.[8,9] Family studies and *JAK2* single nucleotide polymorphism/haplotype analyses suggest genetic predisposition to MPN.[10-12] Although the disease-initiating mutation in MF is not known, the majority of the patients harbors *JAK2*V617F and a minority harbors *MPL*, *LNK*, *CBL*, *TET2*, *ASXL1*, *IDH*, *IKZF1*, or *EZH2* mutations (Table 2).[13,14] These mutations are neither disease-specific nor mutually exclusive[13] and probably constitute secondary events with unpredictable clonal hierarchy.[15] Activating *JAK2* and *MPL* mutations and *LNK* loss-of-function result in constitutive Janus kinase–signal transducer and activator of transcription (JAK-STAT) activation and induce MPN-like disease in mice.[16-19] Therefore, it was appropriate to pursue the therapeutic value of

Table 1. World Health Organization classification of myeloid malignancies and operational subcategorization of myeloproliferative neoplasms

1) Acute myeloid leukemia and related precursor neoplasms

2) Myelodysplastic syndromes (MDS)

3) Myeloproliferative neoplasms (MPN)

　a) Classic MPN

　　i) Chronic myelogenous leukemia (CML), *BCR-ABL1* positive

　　ii) Polycythemia vera (PV)

　　　(1) Chronic phase PV

　　　(2) Post-PV MF

　　　(3) Blast phase PV

　　iii) Essential thrombocythemia (ET)

　　　(1) Chronic phase ET

　　　(2) Post-ET MF

　　　(3) Blast phase ET

　　iv) Primary myelofibrosis (PMF)

　　　(1) Chronic phase PMF

　　　(2) Blast phase PMF

　b) Non-classic MPN

　　i) Chronic neutrophilic leukemia

　　ii) Chronic eosinophilic leukemia, not otherwise specified

　　iii) Mastocytosis

　　iv) Myeloproliferative neoplasm, unclassifiable (MPN-U)

4) MDS/MPN

5) Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA,* * *PDGFRB,* * or *FGFR1* *

*Genetic rearrangements involving platelet-derived growth factor receptor α/β (*PDGFRA/PDGFRB*) or fibroblast growth factor receptor 1 (*FGFR1*).

anti-JAK adenosine triphosphate (ATP) mimetics.[20,21] *TET2, ASXL1,* and *EZH2* mutations are suspected of playing a role in epigenetic dysregulation of transcription.[22-24] *IDH* mutations result in the formation of oncoproteins that might promote disease progression.[25] The individual frequency of the aforementioned mutations in MF (Table 2), save for *JAK2*V617F, is too low to be overexcited about their utility as drug targets.

Clonal myeloproliferation in MF is accompanied by a secondary inflammatory state characterized by bone marrow stromal changes and abnormal cytokine expression.[4] Myeloid cell–derived transforming growth factor-β, platelet-derived growth factor, fibroblast growth factor-b, and vascular endothelial growth factor have all been implicated as mediators of bone marrow fibrosis, osteosclerosis, and angiogenesis in MF.[4] The abnormal release of cytokines, chemokines, and extracellular matrix metalloproteinases (or their inhibitors) might be enhanced by a pathologic cell-cell interaction that involves megakaryocytes, monocytes, and neutrophils[26] and contributes to MF-associated abnormal peripheralization of CD34+ myeloid progenitors[27] and endothelial cells.[28] Plasma levels of proinflammatory cytokines are elevated in MF and might be pathogenetically linked to disease-associated constitutional symptoms and cachexia.[21] In addition, a recent study suggested significant associations between increased interleukin-8 (IL-8), IL-10, IL-15, or IL-2 receptor (IL-2R) and inferior overall survival (OS) and leukemia-free survival in PMF.[29] These observations lend to the possibility that inflammatory mediators might affect survival in MF by either accelerating death from comorbid conditions or promoting clonal evolution.

Diagnosis: How do I diagnose PMF, post-PV/ET MF, and prefibrotic PMF?

Current diagnosis of PMF is based on WHO criteria and includes clinical, morphologic, cytogenetic, and molecular assessments

(Table 3).[30] The diagnosis of post-PV or post-ET MF is according to IWG-MRT criteria (Table 3).[3] In all 3 MF variants, typical laboratory features include anemia (microcytic in ∼ 28% of PMF cases),[31] peripheral blood leukoerythroblastosis, dacryocytosis, leukocytosis/thrombocytosis, increased lactate dehydrogenase (LDH), excess circulating blasts or CD34+ cells, and bone marrow fibrosis, osteosclerosis, and angiogenesis. Occasionally, overt bone marrow fibrosis might be absent (ie, prefibrotic PMF) and, in the presence of thrombocytosis, a spurious diagnosis of ET is made. The possibility of prefibrotic PMF, as opposed to ET, should be considered in the presence of persistently increased serum LDH, anemia, leukoerythroblastosis, increased circulating CD34+ cell count, and marked splenomegaly.[32] It is underscored that the distinction between ET and prefibrotic PMF is clinically relevant because both OS and leukemia-free survival are significantly inferior in the latter.[32] However, there is currently no evidence to suggest a different treatment approach for patients with prefibrotic PMF and thrombocytosis; I manage them the same as if they had ET.

The differential diagnosis of PMF should also include bone marrow fibrosis associated with nonneoplastic or other neoplastic conditions, including metastatic cancer, lymphoid neoplasm, or another myeloid malignancy, especially CML, MDS, chronic myelomonocytic leukemia (CMML), or AML. The presence of *JAK2* or *MPL* mutation, with a combined mutational frequency of ∼ 70%, reliably excludes reactive bone marrow fibrosis or a nonmyeloid malignancy.[33] The absence of *BCR-ABL1* excludes the possibility of CML. MDS or CMML should be considered in the presence of dyserythropoiesis/dysgranulopoiesis or peripheral blood monocytosis ($> 1 \times 10^9$/L), respectively.[34] In this regard, the presence of +9 or 13q− cytogenetic abnormality favors a PMF diagnosis.[35] In contrast, *JAK2*V617F can occur in both MDS and CMML, although infrequently,[36] and is therefore not very useful in distinguishing one myeloid malignancy from another. "Acute myelofibrosis," "acute panmyelosis with myelofibrosis," and "acute megakaryoblastic leukemia" are terms used to describe a biologically aggressive myeloid malignancy that presents acutely with bone marrow fibrosis, pancytopenia, and usually no splenomegaly.[37] Such cases should be managed as AML regardless of which term one uses to describe them.

Risk stratification: prognosis dictates treatment

The International Prognostic Scoring System (IPSS)[38] uses the following 5 risk factors for estimating survival from time of diagnosis: age > 65 years, hemoglobin level < 10 g/dL, leukocyte count $> 25 \times 10^9$/L, circulating blasts ≥ 1%, and presence of constitutional symptoms.[38] The presence of 0, 1, 2, and ≥ 3 adverse factors define low, intermediate 1, intermediate 2, and high-risk disease with median survivals of 11.3, 7.9, 4, and 2.3 years, respectively.[38] With the use of the same prognostic variables, IPSS was later modified to Dynamic IPSS (DIPSS) for use at any time during the disease course.[39] Most recently, DIPSS was upgraded to DIPSS-plus (Figure 1) by the incorporation of 3 additional IPSS/DIPSS independent risk factors, including red cell transfusion need, platelet count $< 100 \times 10^9$/L, and unfavorable karyotype[40]; the latter includes complex karyotype or sole or 2 abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q−, 12p−, or 11q23 rearrangement.[41] The 8 DIPSS-plus risk factors are used to define low (no risk factors), intermediate 1 (1 risk factor), intermediate 2 (2 or 3 risk factors), and high (≥ 4 risk factors) risk

Table 2. Somatic mutations described in classic myeloproliferative neoplasms including primary myelofibrosis, polycythemia vera and essential thrombocythemia

Mutations	Chromosome location	Mutational frequency, %	Pathogenetic relevance
JAK2 (Janus kinase 2)	9p24	PV ~ 96[13]	Contributes to abnormal myeloproliferation and progenitor cell growth factor hypersensitivity[13]
JAK2V617F exon 14 mutation[13]		ET ~ 55[13]	
		PMF ~ 65[13]	
		BP-MPN ~ 50[13]	
JAK2 exon 12 mutation[13]	9p24	PV ~ 3[13]	Contributes to primarily erythroid myeloproliferation[13]
MPL (myeloproliferative leukemia virus oncogene) MPN-associated mutations involve exon 10[13]	1p34	ET ~ 3[13]	Contributes to primarily megakaryocytic myeloproliferation[13]
		PMF ~ 10[13]	
		BP-MPN ~ 5[13]	
TET2 (TET oncogene family member 2) MPN-associated mutations occur across several of the gene's 12 exons[13]	4q24	PV ~ 16[13]	May contribute to epigenetic dysregulation (TET proteins catalyze conversion of 5-methylcytosine to 5-hydroxymethylcytosine) [24]
		ET ~ 5[13]	
		PMF ~ 17[13]	
		BP-MPN ~ 17[13]	
ASXL1 (additional sex combs-like 1) exon 12 mutations	20q11.1	CP-MPN ~ rare[87]	Wild-type ASXL1 is needed for normal hematopoiesis[88] and might be involved in transcriptional repression[23]
		PMF ~ 13[89]	
		BP-MPN ~ 18[89]	
CBL (Casitas B-lineage lymphoma protooncogene) exon 8/9 mutations[90]	11q23.3	PV ~ rare[90]	CBL is an E3 ubiquitin ligase that marks mutant kinases for degradation and transforming activity requires loss of wild-type CBL[91]
		ET ~ rare[90]	
		MF ~ 6[90]	
IDH1/IDH2 (isocitrate dehydrogenase) exon 4 mutations[45]	2q33.3/	PV ~ 2[45]	Induces formation of 2-hydroxyglutarate, a possible oncoprotein[25]
	15q26.1	ET ~ 1[45]	
		PMF ~ 4[45]	
		BP-MPN ~ 20[45]	
IKZF1 (IKAROS family zinc finger 1) (mostly deletions including intragenic[92])	7p12	CP-MPN ~ rare[92]	Transcription regulator and putative tumor suppressor[93]
		BP-MPN ~ 19[92]	
LNK (as in Links) also known as SH2B3 (a membrane-bound adaptor protein) MPN-associated mutations were monoallelic and involved exon 2[18,94]	12q24.12	PV ~ rare[94,95]	Wild-type LNK is a negative regulator of JAK2 signaling[96]
		ET ~ rare[18,94]	
		PMF ~ rare[18,94]	
		BP-MPN ~ 10[94]	
EZH2 (enhancer of zeste homolog 2) both monoallelic and biallelic mutations occur in MPN, involving exons 10, 18, and 20, and are thought to be inactivating[14]	7q36.1	PV ~ 3[14]	Wild-type EZH2 is part of a histone methyltransferase and might function both as a tumor suppressor (myeloid malignancies) [14] and an oncogene (other tumors)
		PMF ~ 7[88]	
		MF ~ 13[14]	

MPN indicates myeloproliferative neoplasms; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; MF includes both PMF and post-ET/PV myelofibrosis; BP-MPN, blast phase MPN; and CP-MPN, chronic phase MPN.

groups with respective median survivals of 15.4, 6.5, 2.9, and 1.3 years (Figure 1).[40] Leukemic transformation was predicted by the presence of unfavorable karyotype or platelet count $< 100 \times 10^9$/L.[40]

The presence or absence of JAK2,[42,43] Ten-Eleven Translocation-2 (TET2),[44] or isocitrate dehydrogenase (IDH)[45] mutations has not been shown to affect either survival or leukemic transformation in PMF. Instead, nullizygosity for JAK2 46/1 haplotype[12,45] and low JAK2V617F allele burden.[42,43] have been shown to be detrimental for survival. Similarly, increased plasma levels of IL-8, IL-10, IL-15, or IL-2R have recently been associated with poor survival that was not accounted for by conventional risk categorization.[29] In other words, our current ability to accurately estimate prognosis in the individual patient with PMF is better than ever and will probably continue to improve with time.

Risk-adapted therapy: Who gets what?

Current drug therapy in PMF is neither curative nor essential for survival. Similarly, it is not clear if the application of allogeneic stem cell transplantation (allo-SCT), with its attendant risk of death

Table 3. Diagnostic criteria

WHO diagnostic criteria for PMF requires meeting all 3 major criteria and ≥ 2 minor criteria outlined[30]
I. Major criteria
a. Megakaryocyte proliferation, including small-to-large megakaryocytes, with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering accompanied by either reticulin and/or collagen fibrosis, *or* in the absence of reticulin fibrosis (ie, prefibrotic PMF), the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (Figure 1).
b. Not meeting WHO criteria for chronic myelogenous leukemia, polycythemia vera, myelodysplastic syndromes, or other myeloid neoplasm
c. Demonstration of *JAK2*V617F or other clonal marker *or* no evidence of reactive marrow fibrosis
II. Minor criteria
a. Leukoerythroblastosis
b. Increased serum lactate dehydrogenase
c. Anemia
d. Palpable splenomegaly
International Working Group for Myeloproliferative Neoplasms Research and Treatment criteria for post-polycythemia vera/essential thrombocythemia (post-PV/ET) myelofibrosis requires meeting both major criteria and ≥ 2 minor criteria[3]
I. Major criteria
a. Documentation of a previous diagnosis of PV or ET as defined by the WHO criteria
b. Bone marrow fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale)*
II. Minor criteria
a. A leukoerythroblastic peripheral blood picture (for both PV and ET)
b. Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥ 5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly (for both PV and ET)
c. Development of ≥ 1 of 3 constitutional symptoms: > 10% weight loss in 6 months, night sweats, unexplained fever (> 37.5°C) (for both PV and ET)
d. Anemia or sustained loss of requirement for phlebotomy in the absence of cytoreductive therapy (for PV)
e. Anemia and a decrease of hemoglobin level ≥ 2 g/dL from baseline (for ET)
f. Increased serum lactate dehydrogenase (for ET)

*Grade 2-3 according to the European classification[97]: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain). Grade 3-4 according to the standard classification[98]: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis.

or chronic morbidity from graft-versus-host disease (GVHD), has had a favorable or unfavorable net effect. Therefore, one must first determine whether a particular patient needs any form of therapy at all and, if so, carefully select the treatment strategy with the best chance of inducing disease control without compromising life expectancy (Figure 2).

How I manage low- or intermediate 1–risk disease

According to the DIPSS-plus prognostic model,[40] the respective median survival of patients with low- or intermediate 1–risk disease exceeds 15 and 6 years and even longer for patients younger than age 65 years. Therefore, the risk of allo-SCT–associated mortality and morbidity is not justified in such patients, and it is also not prudent to subject them to investigational drug therapy, considering the limited information about long-term safety of new therapeutic agents. Similarly, there is no evidence to support the value of conventional drug therapy in asymptomatic patients with low- or intermediate 1–risk disease. Therefore, I prefer a "watch and wait" treatment strategy in such instances, regardless of patient age.

Low-risk patients might occasionally experience symptoms associated with disease manifestations that do not necessarily affect DIPSS-plus risk stratification: splenomegaly, nonhepato-splenic extramedullary hematopoiesis, extramedullary hematopoi-esis (EMH)–associated pulmonary hypertension, fatigue, bone (extremity) pain, pruritus, or thrombocytosis with a thrombosis history. Intermediate 1–risk patients might in addition display symptomatic anemia, marked leukocytosis, or constitutional symp-toms such as drenching night sweats, fever, or weight loss (cachexia). However, such symptoms are much more prevalent in intermediate 2– and high-risk patients, and they are usually not severe enough to warrant therapeutic intervention in lower-risk patients. Nevertheless, if treatment is indicated in low- or interme-diate 1–risk patients, it is reasonable to start with conventional drug therapy (see "Conventional drug therapy") before rushing into treatment with experimental agents.

How I manage intermediate 2– or high-risk disease

Patients with MF with high- or intermediate 2–risk disease can be managed by conventional drug therapy, splenectomy, radiotherapy, allo-SCT, or experimental drug therapy. With each one of these treatment modalities except allo-SCT, the primary goal is palliation of anemia, symptomatic splenomegaly, constitutional symptoms, or disease complications from EMH.

Conventional drug therapy

For the purposes of this review, I define "conventional drugs" as those that are approved by the Food and Drug Administration, and their utility in a specific disease-related complication has been published in a peer-reviewed medical journal. My approach in managing symptomatic anemia depends on the presence or absence of associated splenomegaly. In the absence of splenomegaly, it is reasonable to try erythropoiesis-stimulating agents (ESAs; eg, darbepoetin, 150-300 μg weekly) on the basis of a potential response rate of ≤ 56% (lasting for an average of 1 year) in patients who are not transfusion dependent and show a hemoglobin level of < 10 g/dL.[46] It should be noted, however, that ESAs are unlikely to benefit patients who are transfusion dependent[46] or who display a serum erythropoietin level of > 125 U/L.[47] In addition, the use of ESAs is discouraged in the presence of more than mild splenomegaly (ie, palpable spleen size > 5 cm below the left costal margin), because of the danger of drug-induced exacerbation of splenomegaly.

In anemic patients who are either not good candidates for ESA therapy (see above) or in whom such therapy was unsuccess-ful, one has a choice of several potentially effective conventional drugs, including corticosteroids (eg, prednisone 0.5 mg/kg/d),[48] androgens (eg, fluoxymesterone 10 mg 3 times daily),[49] danazol (600 mg/d),[50] thalidomide (50 mg/d) with or without prednisone (0.25 mg/d)[51,52] or lenalidomide (10 mg/d) with or without predni-sone.[53-55] Response rates and durations for each one of these treatment modalities are somewhat similar and estimated at 20% and 1 year, respectively. Thalidomide or lenalidomide should be
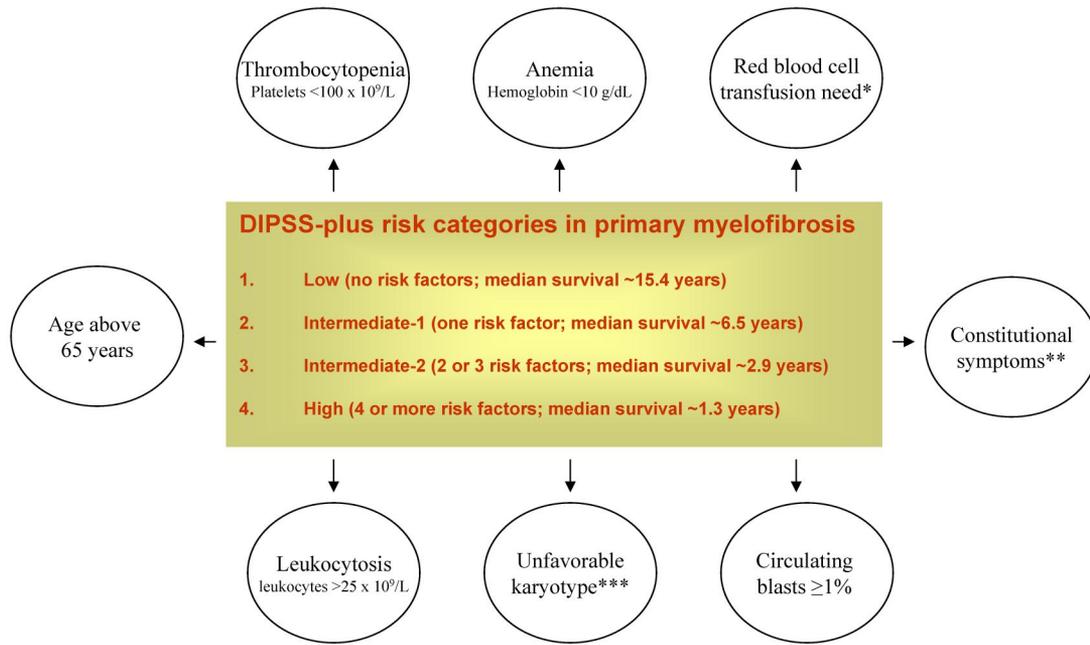
Figure 1. The Dynamic International Prognostic Scoring System (DIPSS) plus prognostic model for primary myelofibrosis (PMF). The DIPSS and prognostic model for PMF uses 8 risk factors for inferior survival: age > 65 years, hemoglobin level < 10 g/dL, leukocyte count > 25 × 10⁹/L, circulating blasts ≥ 1%, presence of constitutional symptoms, presence of unfavorable karyotype, platelet count < 100 × 10⁹/L, and the presence of red cell transfusion need.[40] *Please note that a transfusion-dependent patient automatically has 2 risk factors because of transfusion need (1 risk point) and hemoglobin level < 10 g/dL (1 risk point). **Constitutional symptoms constitute weight loss > 10% of baseline value in the year preceding diagnosis, unexplained fever, or excessive sweats persisting for > 1 month.[38] ***Unfavorable karyotype constitutes complex karyotype or sole or 2 abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q− 12p−, or 11q23 rearrangement.[41]

avoided in women of childbearing age. Corticosteroid use should be avoided in the presence of diabetes or osteopenia; androgen or danazol use should be avoided in the presence of increased level of serum prostate-specific antigen or history of prostate cancer;
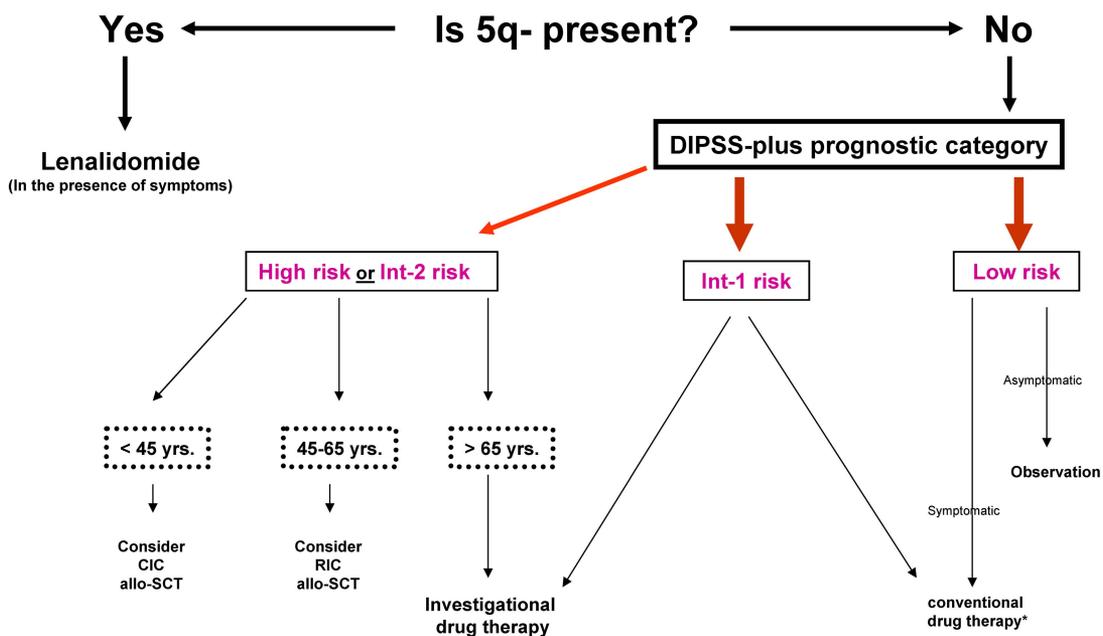

Figure 2. Risk-adapted therapy in primary myelofibrosis. DIPSS-plus indicates Dynamic International Prognostic Scoring System (DIPSS)-plus prognostic model for primary myelofibrosis[40]; Int, intermediate; yrs, years; CIC, conventional-intensity conditioning; RIC, reduced-intensity conditioning; allo-SCT, allogeneic stem cell transplantation. *Conventional drug therapy includes erythropoiesis-stimulating agents, androgens, danazol, corticosteroids, thalidomide, lenalidomide, hydroxyurea, and cladribine. Please see text about which agent is used when.

thalidomide use should be avoided in the presence of neuropathy; and lenalidomide use should be avoided in the presence of moderate-to-severe neutropenia or thrombocytopenia.

I favor the use of lenalidomide in the presence of del(5q) and expect a response in both anemia and splenomegaly.[56] In the absence of del(5q), the therapeutic benefit of lenalidomide, with or without prednisone,[55] is too limited, and its myelosuppressive toxicity too high to warrant its use as first-line therapy. Patients receiving lenalidomide should be closely monitored for the occurrence of severe myelosuppression and thrombosis; I use concomitant aspirin, if the platelet count is $> 50 \times 10^9$/L, to minimize lenalidomide- or thalidomide-associated thrombosis. Thalidomide alone can produce $\sim 20\%$ response rates in MF-associated anemia, thrombocytopenia, or splenomegaly.[52,57] Furthermore, its use in combination with prednisone reduces the severity of its short-term side effects and might enhance its therapeutic activity.[51] Unfortunately, with longer-term usage, a substantial proportion of patients develop thalidomide-associated peripheral neuropathy. Therefore, in the absence of del(5q), I usually try androgens or danazol before resorting to thalidomide; side effects of androgen therapy include hepatotoxicity and virilizing effects.

Hydroxyurea (starting dose 500 mg by mouth twice daily) is my first-line drug of choice for the treatment of MF-associated splenomegaly.[58,59] In the presence of marked splenomegaly (palpable at > 10 cm below the left costal margin), $\sim 35\%$ of patients were reported to achieve $\geq 25\%$ reduction in spleen size; 17% experienced 50% reduction in spleen size.[58] Response rates were significantly lower (10%) in *JAK*2V617F-negative patients, compared with those with detectable *JAK*2V617F: 67% and 33% response rates in patients with mutant allele burdens of $<$ or $> 50\%$, respectively.[58] Spleen responses to hydroxyurea last for an average of 1 year, and treatment side effects include myelosuppression, xerodermia, and mucocutaneous ulcers. At present, participation in clinical trials with JAK inhibitors (see "JAK inhibitors: value and limitations") is advised for patients with hydroxyurea-refractory splenomegaly or hepatomegaly. Otherwise, one can try intravenous cladribine (5 mg/m^2 by 2-hour infusion daily for 5 days and to be repeated monthly, depending on toxicity and response),[60] thalidomide,[52,57] or lenalidomide.[53] Expected response rates for these latter agents range from 20% to 50%. Interferon-α is of limited value in the treatment of MF-associated splenomegaly.[61]

Splenectomy

Splenectomy remains a viable treatment option for drug-refractory symptomatic splenomegaly in MF.[62] In general, I consider splenectomy in the presence of marked splenomegaly (> 10 cm palpable below the left costal margin) that is not responding to adequate doses of hydroxyurea and is associated with severe discomfort or pain, frequent red blood cell transfusions, severe thrombocytopenia, symptomatic portal hypertension, or profound cachexia. Most patients with MF who undergo splenectomy benefit from the procedure; more than one-half of transfusion-dependent patients become transfusion-independent, and most also experience resolution of mechanical symptoms and improvement in constitutional symptoms, cachexia, and platelet count if they were thrombocytopenic.[63] The average duration of response is about a year.

The downside of splenectomy in MF includes a perioperative mortality rate of 5%-10% and morbidity rate of $\sim 25\%$.[62,63] Abdominal vein thrombosis, operative site bleeding, and infections are particularly prevalent during the postoperative period, and close monitoring is critical for early diagnosis and treatment. In preparation for splenectomy, I usually place patients with platelet count of $> 200 \times 10^9$/L on hydroxyurea to minimize the risk of postoperative extreme thrombocytosis and associated thrombosis.[62] Postoperatively and once hemostasis is secured ($\sim$ 5-8 days after surgery), I usually put patients on therapeutic systemic anticoagulation for $\sim$ 1 month to reduce the risk of postoperative splanchnic vein thrombosis. Postsplenectomy thrombocytosis and left-shifted granulocytosis, including an increase in circulating blast percentage, are frequent and do not necessarily imply disease progression. These redistribution changes are often effectively managed by cytoreductive therapy. In addition, $\sim 20\%$ of patients might experience progressive hepatomegaly, and potentially useful drugs for such cases include hydroxyurea, cladribine, or JAK inhibitors (see above in the immediately preceding section). Median survival after splenectomy has been reported to be $\sim$ 2 years. In my opinion, leukemic transformation after splenectomy represents the natural progression of the disease rather than a treatment complication.[64] Alternatives to splenectomy, in hydroxyurea-refractory splenomegaly, include participation in clinical trials, radiotherapy (see "Radiotherapy"), or transjugular intrahepatic portosystemic shunt in case of symptomatic portal hypertension (ie, ascites, recurrent variceal bleed). Because of the lack of adequately sized relevant studies in MF, I do not recommend laparoscopic total or subtotal splenectomy or splenic artery embolization.

Radiotherapy

In MF, radiotherapy is most useful in the setting of non–hepatosplenic EMH,[65] pulmonary hypertension,[66] or lower or upper extremity pain.[67] Non–hepatosplenic EMH might involve the vertebral column (spinal cord compression), lymph nodes (lymphadenopathy), pleura (pleural effusion), peritoneum (ascites), skin (cutaneous nodules), or other tissues and is effectively treated with low-dose radiotherapy (100-500 cGy in 5-10 fractions).[65]

MF-associated pulmonary hypertension is suspected in the presence of clinical symptoms and signs, including dyspnea/hypoxia on exertion and peripheral edema, increased systolic pulmonary artery pressure on echocardiography, and an abnormal pulmonary uptake during a technetium 99m sulphur colloid scintigraphy. It is important to rule out alternative causes such as thromboembolic, infectious, or inflammatory lung processes (high-resolution computed tomographic scanning helps in this regard). In the absence of an alternative explanation for pulmonary hypertension, in a patient with MF, treatment with single-fraction (100 cGy) whole-lung irradiation is reasonable even if the technetium scan was negative.[66]

Single fraction of 100-400 cGy involved field radiotherapy has also been shown to benefit patients with MF-associated extremity pain.[67] Finally, low-dose radiotherapy (100 cGy in 5-10 fractions) can be used in drug-refractory splenomegaly or hepatomegaly and is capable of inducing a transient (3-6 months) reduction in organ size.[68] Such treatment, however, is often associated with severe and sometimes protracted pancytopenia and is best reserved for those patients who are poor surgical candidates for splenectomy and are unable to participate in a clinical trial.[68]

Allogeneic stem cell transplantation

Aside from case reports involving lenalidomide use in patients with del(5q)-associated MF,[56] allo-SCT is currently the only treatment option in MF that is capable of inducing complete hematologic, cytogenetic, and molecular remissions.[69] However, in considering allo-SCT as a treatment modality, one should be acutely aware of the risks involved. In the most recent study from the United

Kingdom,[70] 51 patients with PMF (24%, 33%, and 43% with Dupriez low-, intermediate-, and high-risk disease)[71] received mostly related conventional-intensity conditioning (CIC; ages 19-54 years) or reduced-intensity conditioning (RIC; ages 40-64 years) allo-SCT. Three-year OS was 44% for CIC transplantation and 31% for RIC transplantation, the corresponding relapse rates were 15% and 46%, non-relapse mortality rates were 41% and 32%, and extensive chronic GVHD rates were 30% and 35%.[70]

In an earlier study from the Center for International Bone Marrow Transplant Research involving 289 patients with PMF (ages, 18-73 years; 32%, 36%, and 31% with Dupriez low-, intermediate-, and high-risk disease),[72] treatment-related mortality (TRM) was 27% at 1 year and 35% at 5 years. In the unrelated donor setting, TRM was 43% at 1 year and 50% at 5 years. Five-year OSs were 37% and 30% in related and unrelated donor settings, respectively. Outcome did not appear to be favorably affected by RIC when 3-year disease-free survival (DFS) was 39% and even lower (17%) in the unrelated donor setting.[72] These results were similar to those of a multicenter study of 100 patients from Italy[73] in which RIC transplantation did not affect the 3-year OS of 42% and TRM of 43%. A somewhat higher 5-year disease-free survival (51%) was reported from another RIC allo-SCT study[74] in which relapse was predicted by high-risk disease and prior splenectomy.[74] History of splenectomy did not affect outcome in the Center for International Bone Marrow Transplant Research study.[72]

On the basis of the above, I do not believe that the risk of allo-SCT is currently justified in patients with MF with DIPSS-plus[40] low- or intermediate 1–risk disease patients (Figure 2). I am also not convinced that transplantation-related mortality and morbidity in MF have been favorably altered by the use of RIC transplantation when TRM, relapse, and chronic GVHD rates remain uncomfortably high. Therefore, if allo-SCT is indicated because of high- or intermediate 2–risk disease, I am inclined to favor the use of CIC transplantation in younger patients (age < 40-50 years), considering its association with a lower risk of relapse, compared with RIC transplantation. It is reasonable to offer RIC transplantation for older patients with high- or intermediate 2–risk disease, especially if a matched related donor is available. There is currently no hard data to support the need for splenectomy before transplantation. I prefer alternative means of reducing spleen size such as the use of cytoreductive therapy or JAK inhibitors (see "JAK inhibitors: value and limitations"), although I recognize the unknown effect of the latter agents on outcome after transplantation.

Experimental drug therapy

Investigational drugs in MF include pomalidomide, JAK inhibitor ATP mimetics, histone deacetylase inhibitors (eg, panobinostat, givinostat), and others (eg, hypomethylating agents, bevacizumab, plitidepsin). The most promising among these, so far, are pomalidomide and JAK inhibitors, and each is further elaborated.

Pomalidomide: Is it better than thalidomide or lenalidomide?

Pomalidomide is a thalidomide derivative classified with lenalidomide as an immunomodulatory drug. In vitro, immunomodulatory drugs antagonize angiogenesis and expression of tumor necrosis factor α and IL-6 while they facilitate production of IL-2 and interferon IFN-γ and enhance T-cell and natural killer–cell prolif-

eration and activity; the precise mechanism of their action is not known but might include down-regulation cytokine signaling.[75] All 3 drugs (thalidomide, lenalidomide, and pomalidomide) are active in both multiple myeloma and MF. In MF, thalidomide and lenalidomide, with or without prednisone, have comparable activity in alleviating anemia, splenomegaly, and thrombocytopenia; response rates for each were in the vicinity of 20%.[51-54] Treatment was complicated by peripheral neuropathy or severe myelosuppression in patients receiving thalidomide[52] or lenalidomide,[55] respectively. Therefore, there was room for improvement in both therapeutic activity and side effect profile.

In a phase 2 randomized study, ~ 25% of patients with anemia responded to pomalidomide alone (2 mg/d) or pomalidomide (0.5 or 2 mg/d) combined with prednisone.[48] In a subsequent phase 2 study of single-agent pomalidomide (0.5 mg/d),[76] anemia response was documented only in the presence of *JAK2*V617F (24% vs 0%) and predicted by the presence of pomalidomide-induced basophilia (38% vs 6%) or the absence of marked splenomegaly (38% vs 11%). Platelet response was seen in 58% of patients with baseline platelet count of 50-100 $\times$ 10^9/L, but the drug had limited activity in reducing spleen size.[76] Unlike the case with thalidomide or lenalidomide, drug-associated neuropathy or myelosuppression was infrequent. However, higher doses of pomalidomide (> 2 mg/d) were myelosuppressive and not necessarily better in terms of efficacy.

In choosing between thalidomide, lenalidomide, and pomalidomide, I prefer to use lenalidomide in the presence of del(5q), because of the possibility of obtaining hematologic and cytogenetic remissions.[56] In the absence of del(5q), my decision hinges on the presence or absence of *JAK2*V617F or marked splenomegaly. My choice is pomalidomide for the *JAK2*V617F-positive patient without marked splenomegaly.[76] Otherwise, it is reasonable to give thalidomide plus prednisone a try.[51] Of note, I would not use any of these 3 drugs in the absence of symptomatic anemia.

JAK inhibitors: value and limitations

JAK2 inhibitor ATP mimetics that are currently in clinical trials include INCB018424, TG101348, CEP-701, CYT387, AZD1480, SB1518, and LY2784544 (http://www.clinicaltrials.gov; Table 4). Results of these studies so far suggest substantial differences among these drugs in their toxicity and efficacy profiles, some of which might be linked to their pharmacokinetic properties and variable in vitro activity against other JAK and non-JAK kinase targets.

INCB018424 is a JAK1/JAK2 inhibitor. In a phase 1/2 study of 153 patients with MF,[21] dose-limiting toxicity was thrombocytopenia, and the maximum tolerated dose was either 25 mg twice daily or 100 mg once daily. Adverse events included thrombocytopenia, anemia, and a "cytokine rebound reaction" on drug discontinuation. The latter is characterized by acute relapse of symptoms and splenomegaly, sometimes necessitating hospitalization. In a recent publication, 2 patients (1.3%) were reported to have experienced a systemic inflammatory response syndrome on drug discontinuation. To prevent or decrease the intensity of cytokine rebound reaction, I avoid abrupt drug discontinuation and instead use a 2-week tapering schedule. In addition, I sometimes use oral corticosteroid therapy (0.5 mg/kg/d), again in a tapering schedule, to help patients tolerate the event. Grade 3/4 thrombocytopenia or anemia in previously nontransfused patients occurred in 39% and 43% of patients receiving the drug at the recommended dose of 25 or 10 mg twice daily. At the lower dose level of 10 mg twice

Table 4. JAK2 inhibitors that have made it to clinical trials in myelofibrosis and not terminated as yet

Anti-JAK2 ATP mimetic	Anti-JAK2 IC50 (JAK1/JAK3/TYK2 selectivity)	Non-JAK kinase targets	Clinical trials	Disease features shown to be favorably affected	Side effects
INCB018424[21] (phase 1/2 study)	5.7nM (×1.0/×98/×9.3)	None of ~ 28 kinases evaluated	MF (n = 153) [21]	Splenomegaly, constitutional symptoms, pruritus, cachexia	Thrombocytopenia (DLT) anemia, acute relapse of symptoms and re-enlargement of spleen on drug discontinuation, SIRS on drug discontinuation"
TG101348[20] (phase 1/2 study)	3nM (×35/×332/×135)	FLT3 RET	MF (n = 59)	Splenomegaly, constitutional symptoms, pruritus, leukocytosis, thrombocytosis, JAK2V617F burden	Increased amylase/lipase (DLT), anemia, thrombocytopenia, nausea/vomiting, diarrhea, increased transaminases
CEP-701[77] (lestaurtinib) (phase 2 study)	1nM (×?/×3/×?)	FLT3 TrkA	MF (n = 22)	Splenomegaly, anemia, pruritus	Diarrhea, nausea/vomiting, anemia, thrombocytopenia
CYT387[78] (phase 1/2 study)	18nM (×0.6/×8.6/×?)	JNK1 CDK2	MF (n = 36)	Anemia, splenomegaly, constitutional symptoms, pruritus	Increased amylase/lipase (DLT), headache (DLT), thrombocytopenia, increased, transaminases, "first dose-effect characterized by transient hypotension and lightheadedness"
AZD1480[99] (phase 1/2 study)	0.26nM (×5/×15/×?)	TrkA Aurora A FGFR1	MF	Results pending	Results pending
SB1518[100] (phase 1/2 study)	22nM (×58/×24/×?)	FLT3	MF (n = 31)	Splenomegaly	(DLT = GI symptoms), diarrhea, nausea thrombocytopenia
LY2784544 (phase 1/2 study)	Scant literature	Scant literature	MF	Results pending	Results pending

MF indicates myelofibrosis and includes PMF and post-PV/ET MF; DLT, dose-limiting toxicity; SIRS, systemic inflammatory response syndrome; and GI, gastrointestinal.

daily, the corresponding figures were 10% and 16%, but the response rate for splenomegaly (30%) was also lower at 10 mg twice daily compared with 25 mg twice daily (49%).[21] Among all evaluable patients, 44% experienced ≥ 50% decrease in palpable spleen size. Improvement in constitutional symptoms (fatigue, pruritus, abdominal discomfort, early satiety, night sweats) and weight gain were seen in the majority of patients. Four of 28 transfusion-dependent patients (14%) became transfusion-independent. The drug's effect on JAK2V617F allele burden or bone marrow pathology was negligible, but a major reduction in proinflammatory cytokines (eg, IL-1RA, IL-6, tumor necrosis factor-α, macrophage-inflammatory protein-1β) was documented and coincided with improvement in constitutional symptoms.

TG101348 is a JAK2/fms-like tyrosine kinase 3 inhibitor. In a phase 1/2 study of 59 patients with MF,[20] dose-limiting toxicity was a reversible and asymptomatic increase in serum amylase and lipase, and maximum tolerated dose was 680 mg/d. Grade 3/4 adverse events were all reversible and dose-dependent and included nausea (3%); vomiting (3%); diarrhea (10%); asymptomatic increases in serum lipase (27%), transaminases (27%) or creatinine (24%); thrombocytopenia (24%); and anemia (35%). After 6 and 12 months of treatment, 39% and 47% of patients, respectively, achieved a ≥ 50% decrease in palpable spleen size, and the majority also experienced improvement in early satiety, fatigue, night sweats, cough, or pruritus. Almost all patients with thrombocytosis and the majority with leukocytosis had drug-induced normalization of their counts. In general, responses were not affected by the presence or absence of JAK2V617F; however, a > 50% decrease in mutant allele burden was seen in 39% of patients with baseline JAK2V617F allele burden of > 20%. Effect on bone marrow pathology or plasma cytokine levels was unremarkable.

CEP-701 is a JAK2/fms-like tyrosine kinase 3 inhibitor. In a phase 2 study, 22 patients with JAK2V617F-positive MF received

the drug orally at 80 mg twice daily.[77] A > 50% spleen reduction was achieved in 4 of 18 evaluable patients (22%), and 2 of 8 transfusion-dependent patients (25%) became transfusion independent.[77] Grade 3/4 side effects included anemia (14%), thrombocytopenia (23%), and diarrhea (9%). The drug did not affect bone marrow fibrosis or JAK2V617F allele burden. Nineteen different plasma cytokines, including proinflammatory cytokines, were measured at baseline and after treatment and showed no significant differences in their levels between responders and nonresponders and between baseline and posttreatment samples.

Results from other clinical trials of JAK inhibitors have not been published in full. However, glancing at the 2010 American Society of Hematology meeting abstracts shows encouraging preliminary observations from a phase 1/2 study that used another JAK1/JAK2 inhibitor, CYT387, in which an impressive 40% response rate in anemia accompanied equally remarkable response rates in splenomegaly and constitutional symptoms.[78] The drug was well tolerated with infrequent grade 3/4 adverse events that included thrombocytopenia in 22% of patients and anemia in only 3%. Information about other JAK inhibitors (AZD1480, SB1518, and LY2784544) was either less impressive or not available.

On the basis of this discussion, 3 major points can be made about currently available JAK inhibitors and their value in MF. First, none of them were capable of inducing complete or partial remissions, but they definitely have palliative value. Second, they are significantly different from each other in terms of both therapeutic activity and side effect profile. The third point concerns their mechanism of action, which might involve down-regulation of proinflammatory cytokines for INCB018424 and clonal myeloproliferation for TG101348. It is possible that the balance of their composite effect on these parameters governs the spectrum of their activity. In my opinion, the therapeutically most-promising JAK inhibitors at this point are CYT387, TG101348, and

INCB018424, but longer follow-up is needed to fully appreciate their safety profile.

The future: yes we can, but…

None of the currently known mutations in *BCR-ABL1*–negative MPN exhibit *BCR-ABL1*–like disease specificity or pathogenetic significance. However, the remarkably high *JAK2* mutational frequency in these diseases justifies the flurry of current activity in preclinical and clinical evaluation of drugs that target JAK-STAT. I do not foresee a therapeutic success such as imatinib and CML anytime soon because molecular pathogenesis in MF is complex and involves multiple mutations and aberrant pathways. It is possible that these putatively secondary changes are derived from a common mutant stem cell with a specific disease-causing mutation. It is also possible that MF is not one but many diseases with different molecular signatures. Either way, it is becoming more and more evident that JAK inhibitors, by themselves, may not constitute adequate therapy in MF and have yet to show disease-modifying activity. Whether this will change by refining drug specificity to mutant as opposed to wild-type JAK remains to be seen. Nevertheless, JAK inhibitors have unquestionable palliative value,[20,21] and our challenge in this regard is to figure out which JAK inhibitor is appropriate for which disease, which patient, and at what point in the disease course. There is also room for improvement in terms of both therapeutic activity and side effect profile; for example, in addition to the now well-established value of certain JAK inhibitors (eg INCB018424, TG101348)[20,21] in reducing spleen size and alleviating constitutional symptoms, preliminary results suggest that CYT387, a newer JAK1/2 inhibitor, also induces anemia response in a substantial proportion of patients.[78]

Drug-induced complete remissions are possible in MF, as has been previously shown for lenalidomide, in the presence of isolated del(5q).[56] It is therefore critical to continue laboratory investigations and new drug development with this goal in mind and not be content with what we have accomplished so far with JAK inhibitors.[20,21] In this regard, phosphatidylinositol-3 kinase/Akt/mammalian target of rapamycin, Ras/mitogen-activated protein kinase and extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase, STAT3/5, and heat shock protein 90 are alternative MPN-relevant drug targets with some encouraging preclinical[79-82] and clinical[82] data. There is also an obvious interest in combining these drugs with each other[83] or with conventional drugs such as hydroxyurea or ESAs. I am also intrigued by the significant antianemia activity of pomalidomide in *JAK2*V617F-positive MF.[76] However, I have, thus far, not been impressed by the net value (benefit minus risk) of histone deacetylase inhibitors or hypomethylating agents.[84-86] Finally, the recent demonstration of a correlation between JAK inhibitor therapy-induced down-regulation of proinflammatory cytokines and clinical benefit in MF,[21] combined with new information on specific plasma cytokines in PMF and their DIPSS-plus–independent prognostic relevance,[29] supports further evaluation of targeted anticytokine therapy.

Authorship

Contribution: A.T. was the single author of this paper.

Conflict-of-interest disclosure: The author declares no competing financial interests. He serves as principal investigator or co-investigator on many clinical trials, including those that are industry-sponsored. The latter include pomalidomide (Celgene), INCB018424 (Incyte), TG101348 (Targegene), CYT387 (YM Biosciences), and panobinostat (Novartis).

Correspondence: Ayalew Tefferi, Division of Hematology, Department of Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: tefferi.ayalew@mayo.edu.

References

1. Kuter DJ, Bain B, Mufti G, Bagg A, Hasserjian RP. Bone marrow fibrosis: pathophysiology and clinical significance of increased bone marrow stromal fibres. *Br J Haematol.* 2007;139(3):351-362.

2. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood.* 2009;114(5):937-951.

3. Barosi G, Mesa RA, Thiele J, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment. *Leukemia.* 2008;22(2):437-438.

4. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. *J Clin Oncol.* 2005;23(33):8520-8530.

5. Mesa RA, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). *Leuk Res.* 2007;31(6):737-740.

6. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood.* 1951;6:372-375.

7. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia.* 2008;22(1):14-22.

8. Lambert JR, Everington T, Linch DC, Gale RE. In essential thrombocythemia, multiple JAK2-V617F clones are present in most mutant-positive patients: a new disease paradigm. *Blood.* 2009;114(14):3018-3023.

9. Beer PA, Jones AV, Bench AJ, et al. Clonal diversity in the myeloproliferative neoplasms: independent origins of genetically distinct clones. *Br J Haematol.* 2009;144(6):904-908.

10. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. *Blood.* 2008;112(6):2199-2204.

11. Pardanani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. *Blood.* 2008;111(5):2785-2789.

12. Tefferi A, Lasho TL, Patnaik MM, et al. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. *Leukemia.* 2010;24(1):105-109.

13. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia.* 2010;24(6):1128-1138.

14. Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet.* 2010; 42(8):722-726.

15. Schaub FX, Looser R, Li S, et al. Clonal analysis of TET2 and JAK2 mutations suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. *Blood.* 2010;115(10): 2003-2007.

16. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005; 434(7037):1144-1148.

17. Pikman Y, Lee BH, Mercher T, et al. MPLW515L Is a Novel Somatic Activating Mutation in Myelofibrosis with Myeloid Metaplasia. *PLoS Med.* 2006; 3(7):e270.

18. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood.* 2010;116(6):988-992.

19. Bersenev A, Wu C, Balcerek J, et al. Lnk constrains myeloproliferative diseases in mice. *J Clin Invest.* 2010;120(6):2058-2069.

20. Pardanani A, Gotlib JR, Jamieson C, et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. *J Clin Oncol.* Prepublished on December 13, 2010, as DOI 10.1200/ JCO.2010.32.2446.

21. Verstovsek S, Kantarjian H, Mesa RA, et al. Safety and Efficacy of INCB018424, a JAK1 and

From bloodjournal.hematologylibrary.org by guest on April 11, 2011. For personal use only.

BLOOD, 31 MARCH 2011 • VOLUME 117, NUMBER 13

HOW I TREAT MYELOFIBROSIS   3503

JAK2 Inhibitor, in Myelofibrosis. *N Engl J Med.* 2010;363(12):1117-1127.

22. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science.* 2009;324(5929):930-935.

23. Lee SW, Cho YS, Na JM, et al. ASXL1 represses retinoic acid receptor-mediated transcription through associating with HP1 and LSD1. *J Biol Chem.* 2010;285(1):18-29.

24. Ko M, Huang Y, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature.* 2010;468(7322): 839-843.

25. Ward PS, Patel J, Wise DR, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell.* 2010;17(3):225-234.

26. Schmitt A, Jouault H, Guichard J, Wendling F, Drouin A, Cramer EM. Pathologic interaction between megakaryocytes and polymorphonuclear leukocytes in myelofibrosis. *Blood.* 2000;96(4): 1342-1347.

27. Cho SY, Xu M, Roboz J, Lu M, Mascarenhas J, Hoffman R. The effect of CXCL12 processing on CD34+ cell migration in myeloproliferative neoplasms. *Cancer Res.* 2010;70(8):3402-3410.

28. Massa M, Rosti V, Ramajoli I, et al. Circulating CD34+, CD133+, and vascular endothelial growth factor receptor 2-positive endothelial progenitor cells in myelofibrosis with myeloid metaplasia. *J Clin Oncol.* 2005;23(24):5688-5695.

29. Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A, Tefferi A. Circulating IL-2R, IL-8, IL-15 and CXCL10 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study [abstract]. *Blood.* 2010; 116(21):Abstract 3068.

30. Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood.* 2007;110(4):1092-1097.

31. Tefferi A, Dingli D, Li CY, Mesa RA. Microcytosis in agnogenic myeloid metaplasia: prevalence and clinical correlates. *Leuk Res.* 2006;30(6):677-680.

32. Barbui T, Thiele J, Passamonti F, et al. Survival and risk of leukemic transformation in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study on 1104 patients [abstract]. *Blood.* 2010; 116(21):Abstract 457.

33. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. *Leukemia.* 2008;22(7):1299-1307.

34. Steensma DP, Hanson CA, Letendre L, Tefferi A. Myelodysplasia with fibrosis: a distinct entity? *Leuk Res.* 2001;25(10):829-838.

35. Bacher U, Schnittger S, Kern W, Weiss T, Haferlach T, Haferlach C. Distribution of cytogenetic abnormalities in myelodysplastic syndromes, Philadelphia negative myeloproliferative neoplasms, and the overlap MDS/MPN category. *Ann Hematol.* 2009;88(12):1207-1213.

36. Steensma DP, Dewald GW, Lasho TL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. *Blood.* 2005;106(4):1207-1209.

37. Orazi A, O'Malley DP, Jiang J, et al. Acute panmyelosis with myelofibrosis: an entity distinct from acute megakaryoblastic leukemia. *Mod Pathol.* 2005;18(5):603-614.

38. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood.* 2009;113(13):2895-2901.

39. Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood.* 2010;115(9):1703-1708.

40. Gangat N, Caramazza D, Vaidya R, et al. DIPSS-Plus: a refined Dynamic International Prognostic Scoring System (DIPSS) for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count and transfusion status. *J Clin Oncol.* 2011;29(4):392-397.

41. Caramazza D, Begna KH, Gangat N, et al. Refined cytogenetic risk categorization for overall and leukemia-free survival in primary myelofibrosis: A single center study of 433 patients. *Leukemia.* 2011;25(1):82-88.

42. Tefferi A, Lasho TL, Huang J, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. *Leukemia.* 2008; 22(4):756-761.

43. Guglielmelli P, Barosi G, Specchia G, et al. Identification of patients with poorer survival in primary myelofibrosis based on the burden of JAK2V617F mutated allele. *Blood.* 2009;114(8):1477-1483.

44. Tefferi A, Pardanani A, Lim KH, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. *Leukemia.* 2009;23(5):905-911.

45. Tefferi A, Lasho TL, Abdel-Wahab O, et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia.* 2010;24(7):1302-1309.

46. Huang J, Tefferi A. Erythropoiesis stimulating agents have limited therapeutic activity in transfusion-dependent patients with primary myelofibrosis regardless of serum erythropoietin level. *Eur J Haematol.* 2009;83(2):154-155.

47. Cervantes F, Alvarez-Larran A, Hernandez-Boluda JC, Sureda A, Torrebadell M, Montserrat E. Erythropoietin treatment of the anaemia of myelofibrosis with myeloid metaplasia: results in 20 patients and review of the literature. *Br J Haematol.* 2004;127(4):399-403.

48. Tefferi A, Verstovsek S, Barosi G, et al. Pomalidomide is active in the treatment of anemia associated with myelofibrosis. *J Clin Oncol.* 2009; 27(27):4563-4569.

49. Besa EC, Nowell PC, Geller NL, Gardner FH. Analysis of the androgen response of 23 patients with agnogenic myeloid metaplasia: the value of chromosomal studies in predicting response and survival. *Cancer.* 1982;49(2):308-313.

50. Cervantes F, Alvarez-Larran A, Domingo A, Arellano-Rodrigo E, Montserrat E. Efficacy and tolerability of danazol as a treatment for the anaemia of myelofibrosis with myeloid metaplasia: long-term results in 30 patients. *Br J Haematol.* 2005;129(6):771-775.

51. Mesa RA, Steensma DP, Pardanani A, et al. A phase 2 trial of combination low-dose thalidomide and prednisone for the treatment of myelofibrosis with myeloid metaplasia. *Blood.* 2003;101(7): 2534-2541.

52. Thomas DA, Giles FJ, Albitar M, et al. Thalidomide therapy for myelofibrosis with myeloid metaplasia. *Cancer.* 2006;106(9):1974-1984.

53. Tefferi A, Cortes J, Verstovsek S, et al. Lenalidomide therapy in myelofibrosis with myeloid metaplasia. *Blood.* 2006;108(4):1158-1164.

54. Quintas-Cardama A, Kantarjian HM, Manshouri T, et al. Lenalidomide plus prednisone results in durable clinical, histopathologic, and molecular responses in patients with myelofibrosis. *J Clin Oncol.* 2009;27(28):4760-4766.

55. Mesa RA, Yao X, Cripe LD, et al. Lenalidomide and prednisone for myelofibrosis: Eastern Cooperative Oncology Group (ECOG) phase 2 trial E4903. *Blood.* 2010;116(22):4436-4438.

56. Tefferi A, Lasho TL, Mesa RA, Pardanani A, Ketterling RP, Hanson CA. Lenalidomide therapy in del(5)(q31)-associated myelofibrosis: cytogenetic and JAK2V617F molecular remissions. *Leukemia.* 2007;21(8):1827-1828.

57. Marchetti M, Barosi G, Balestri F, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial. *J Clin Oncol.* 2004;22(3):424-431.

58. Siragusa S, Vaidya R, Tefferi A. Hydroxyurea effect on marked splenomegaly associated with primary myelofibrosis: response rates and correlation with JAK2V617F allele burden [abstract]. *Blood.* 2009;114(22):Abstract 4971.

59. Martinez-Trillos A, Gaya A, Maffioli M, et al. Efficacy and tolerability of hydroxyurea in the treatment of the hyperproliferative manifestations of myelofibrosis: results in 40 patients. *Ann Hematol.* 2010;89(12):1233-1237.

60. Faoro LN, Tefferi A, Mesa RA. Long-term analysis of the palliative benefit of 2-chlorodeoxyadenosine for myelofibrosis with myeloid metaplasia. *Eur J Haematol.* 2005;74(2):117-120.

61. Tefferi A, Elliot MA, Yoon SY, et al. Clinical and bone marrow effects of interferon alfa therapy in myelofibrosis with myeloid metaplasia [letter]. *Blood.* 2001;97(6):1896.

62. Tefferi A, Mesa RA, Nagorney DM, Schroeder G, Silverstein MN. Splenectomy in myelofibrosis with myeloid metaplasia: a single-institution experience with 223 patients. *Blood.* 2000;95(7):2226-2233.

63. Mesa RA, Nagorney DS, Schwager S, Allred J, Tefferi A. Palliative goals, patient selection, and perioperative platelet management: outcomes and lessons from 3 decades of splenectomy for myelofibrosis with myeloid metaplasia at the Mayo Clinic. *Cancer.* 2006;107(2):361-370.

64. Barosi G, Ambrosetti A, Centra A, et al. Splenectomy and risk of blast transformation in myelofibrosis with myeloid metaplasia. Italian Cooperative Study Group on Myeloid with Myeloid Metaplasia. *Blood.* 1998;91(10):3630-3636.

65. Koch CA, Li CY, Mesa RA, Tefferi A. Nonhepatosplenic extramedullary hematopoiesis: associated diseases, pathology, clinical course, and treatment. *Mayo Clin Proc.* 2003;78(10):1223-1233.

66. Steensma DP, Hook CC, Stafford SL, Tefferi A. Low-dose, single-fraction, whole-lung radiotherapy for pulmonary hypertension associated with myelofibrosis with myeloid metaplasia. *Br J Haematol.* 2002;118(3):813-816.

67. Neben-Wittich MA, Brown PD, Tefferi A. Successful treatment of severe extremity pain in myelofibrosis with low-dose single-fraction radiation therapy. *Am J Hematol.* 2010;85(10):808-810.

68. Elliott MA, Tefferi A. Splenic irradiation in myelofibrosis with myeloid metaplasia: a review. *Blood Rev.* 1999;13(3):163-170.

69. Alchalby H, Badbaran A, Zabelina T, et al. Impact of JAK2V617F mutation status, allele burden, and clearance after allogeneic stem cell transplantation for myelofibrosis. *Blood.* 2010;116(18):3572-3581.

70. Stewart WA, Pearce R, Kirkland KE, et al. The role of allogeneic SCT in primary myelofibrosis: a British Society for Blood and Marrow Transplantation study. *Bone Marrow Transplant.* 2010;45(11): 1587-1593.

71. Dupriez B, Morel P, Demory JL, et al. Prognostic factors in agnogenic myeloid metaplasia: a report on 195 cases with a new scoring system. *Blood.* 1996;88(3):1013-1018.

72. Ballen KK, Shrestha S, Sobocinski KA, et al. Outcome of transplantation for myelofibrosis. *Biol Blood Marrow Transplant.* 2010;16(3):358-367.

73. Patriarca F, Bacigalupo A, Sperotto A, et al. Allogeneic hematopoietic stem cell transplantation in myelofibrosis: the 20-year experience of the Gruppo Italiano Trapianto di Midollo Osseo (GITMO). *Haematologica.* 2008;93(10):1514-1522.

74. Kroger N, Holler E, Kobbe G, et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Blood.* 2009; 114(26):5264-5270.

75. Gorgun G, Calabrese E, Soydan E, et al. Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. *Blood.* 2010;116(17):3227-3237.

76. Begna KH, Mesa RA, Pardanani A, et al. A phase-2 trial of low-dose pomalidomide in myelofibrosis with anemia. *Leukemia.* 2011;25(2):301-304.

77. Santos FP, Kantarjian HM, Jain N, et al. Phase 2 study of CEP-701, an orally available JAK2 inhibitor, in patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. *Blood.* 2010;115(6):1131-1136.

78. Pardanani A, George G, Lasho T, et al. A phase I/II study of CYT387, an oral JAK-1/2 inhibitor, in myelofibrosis: significant response rates in anemia, splenomegaly, and constitutional symptoms [abstract]. *Blood.* 2010;116(21):Abstract 460.

79. Oku S, Takenaka K, Kuriyama T, et al. JAK2 V617F uses distinct signalling pathways to induce cell proliferation and neutrophil activation. *Br J Haematol.* 2010;150(3):334-344.

80. Marubayashi S, Koppikar P, Taldone T, et al. HSP90 is a therapeutic target in JAK2-dependent myeloproliferative neoplasms in mice and humans. *J Clin Invest.* 2010;120(10):3578-3593.

81. Li G, Miskimen KL, Wang Z, et al. Effective targeting of STAT5-mediated survival in myeloproliferative neoplasms using ABT-737 combined with rapamycin. *Leukemia.* 2010;24(8):1397-1405.

82. Vannucchi AM, Guglielmelli P, Gattoni E, et al. RAD001, an inhibitor of mTOR, shows clinical activity in a phase I/II study in patients with primary myelofibrosis (PMF) and post polycythemia vera/essential thrombocythemia myelofibrosis (PPV/PET MF) [abstract]. *Blood.* 2009;114(22): Abstract 307.

83. Wang Y, Fiskus W, Chong DG, et al. Cotreatment with panobinostat and JAK2 inhibitor TG101209 attenuates JAK2V617F levels and signaling and exerts synergistic cytotoxic effects against human myeloproliferative neoplastic cells. *Blood.* 2009; 114(24):5024-5033.

84. Quintas-Cardama A, Tong W, Kantarjian H, et al. A phase II study of 5-azacitidine for patients with primary and post-essential thrombocythemia/ polycythemia vera myelofibrosis. *Leukemia.* 2008;22(5):965-970.

85. Rambaldi A, Dellacasa CM, Finazzi G, et al. A pilot study of the Histone-Deacetylase inhibitor Givinostat in patients with JAK2V617F positive chronic myeloproliferative neoplasms. *Br J Haematol.* 2010;150(4):446-455.

86. DeAngelo DJ, Spencer A, Fischer T, et al. Activity of oral panobinostat (LBH589) in patients with myelofibrosis [abstract]. *Blood.* 2009;114(22): Abstract 2898.

87. Carbuccia N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia.* 2009;23(11):2183-2186.

88. Fisher CL, Pineault N, Brookes C, et al. Loss-of-function Additional sex combs like 1 mutations disrupt hematopoiesis but do not cause severe myelodysplasia or leukemia. *Blood.* 2010;115(1): 38-46.

89. Abdel-Wahab O, Pardanani A, Patel J, et al. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms [abstract]. *Blood.* 2010;116(21):Abstract 3070.

90. Grand FH, Hidalgo-Curtis CE, Ernst T, et al. Frequent CBL mutations associated with 11q ac-

quired uniparental disomy in myeloproliferative neoplasms. *Blood.* 2009;113(24):6182-6192.

91. Sanada M, Suzuki T, Shih LY, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature.* 2009;460(7257): 904-908.

92. Jager R, Gisslinger H, Passamonti F, et al. Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. *Leukemia.* 2010; 24(7):1290-1298.

93. Trageser D, Iacobucci I, Nahar R, et al. Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. *J Exp Med.* 2009;206(8):1739-1753.

94. Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia.* 2010;24(10):1713-1718.

95. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. *N Engl J Med.* 2010;363(12):1189-1190.

96. Gery S, Cao Q, Gueller S, Xing H, Tefferi A, Koeffler HP. Lnk inhibits myeloproliferative disorder-associated JAK2 mutant, JAK2V617F. *J Leukoc Biol.* 2009;85(6):957-965.

97. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica.* 2005;90(8):1128-1132.

98. Manoharan A, Horsley R, Pitney WR. The reticulin content of bone marrow in acute leukaemia in adults. *Br J Haematol.* 1979;43(2):185-190.

99. Hedvat M, Huszar D, Herrmann A, et al. The JAK2 inhibitor AZD1480 potently blocks Stat3 signaling and oncogenesis in solid tumors. *Cancer Cell.* 2009;16(6):487-497.

100. Verstovsek S, Odenike O, Scott B, et al. Phase I dose-escalation trial of SB1518, a novel JAK2/ FLT3 inhibitor, in acute and chronic myeloid diseases, including primary or post-essential thrombocythemia/ polycythemia vera myelofibrosis [abstract]. *Blood.* 2009;114(22):Abstract 3905.